METHODS AND COMPOSITIONS FOR THE TREATMENT OF PANCREATITIS

This application is a continuation-in-part of application serial no. 09/288,326, filed April 8, 1999.

Field of the Invention

The present invention includes methods and compositions for the treatment of acute pancreatitis. In a preferred embodiment the invention concerns the use of agents to reduce or prevent the secretion of pancreatic digestive enzymes within the pancreas. Such agents are targeted to pancreatic cells, and serve to prevent the exocytotic fusion of vesicles containing these enzymes with the plasma membrane. The invention is also concerned with methods of treating a mammal suffering from pancreatitis through the administration of such agents.

Background of the Invention

Pancreatitis is a serious medical condition involving an inflammation of the pancreas. In acute or chronic pancreatitis the inflammation manifests itself in the release and activation of pancreatic enzymes within the organ itself, leading to autodigestion. In many cases of acute pancreatitis, the condition can lead to death.

In normal mammals, the pancreas, a large gland similar in structure to the salivary gland, is responsible for the production and secretion of

digestive enzymes, which digest ingested food, and bicarbonate for the neutralization of the acidic chyme produced in the stomach. The pancreas contains acinar cells, responsible for enzyme production, and ductal cells, which secrete large amounts of sodium bicarbonate solution. The combined secretion product is termed "pancreatic juice"; this liquid flows through the pancreatic duct past the sphincter of Oddi into the duodenum. The secretion of pancreatic juice is stimulated by the presence of chyme in the upper portions of the small intestine, and the precise composition of pancreatic juice appears to be influenced by the types of compounds (carbohydrate, lipid, protein, and/or nucleic acid) in the chyme.

The constituents of pancreatic juice includes proteases (trypsin, chymotrypsin, carboxypolypeptidase), nucleases (RNAse and DNAse), pancreatic amylase, and lipases (pancreatic lipase, cholesterol esterase and phospholipase). Many of these enzymes, including the proteases, are initially synthesized by the acinar cells in an inactive form as zymogens: thus trypsin is synthesized as trypsinogen, chymotrypsin as chymotypsinogen, and carboxypolypeptidase as procarboxypolypeptidase. These enzymes are activated according to a cascade, wherein, in the first step, trypsin is activated through proteolytic cleavage by the enzyme enterokinase. Trypsinogen can also be autoactivated by trypsin; thus one activation has begun, the activation process can proceed rapidly. Trypsin, in turn, activates both chymotypsinogen and procarboxypolypeptidase to form their active protease counterparts.

The enzymes are normally activated only when they enter the intestinal mucosa in order to prevent autodigestion of the pancreas. In order to prevent premature activation, the acinar cells also co-secrete a trypsin inhibitor that normally prevents activation of the proteolytic enzymes within the secretory cells and in the ducts of the pancreas. Inhibition of trypsin activity also prevents activation of the other proteases.

Pancreatitis can occur when an excess amount of trypsin saturates the supply of trypsin inhibitor. This, in turn, can be caused by underproduction of trypsin inhibitor, or the overabundance of trypsin within the cells or ducts of the pancreas. In the latter case, pancreatic trauma or blockage of a duct can lead to localized overabundance of trypsin; under acute conditions large amounts of pancreatic zymogen secretion can pool in the damaged areas of the pancreas. If even a small amount of free trypsin is available activation of all the zymogenic proteases rapidly occurs, and can lead to digestion of the pancreas (acute pancreatitis) and in particularly severe cases to the patient's death.

Pancreatic secretion is normally regulated by both hormonal and nervous mechanisms. When the gastric phase of stomach secretion occurs, parasympathetic nerve impulses are relayed to the pancreas, which initially results in acetylcholine release, followed by secretion of enzymes into the pancreatic acini for temporary storage.

When acid chyme thereafter enters the small intestine, the mucosal cells of the upper intestine release a hormone called secretin. In humans, secretin

is a 27 amino acid (3400 Dalton) polypeptide initially produced as the inactive form prosecretin, which is then activated by proteolytic cleavage. Secretin is then absorbed into the blood. Secretin causes the pancreas to secrete large quantities of a fluid containing bicarbonate ion. Secretin does not stimulate the acinar cells, which produce the digestive enzymes. The bicarbonate fluid serves to neutralize the chyme and to provide a slightly alkaline optimal environment for the enzymes.

Another peptide hormone, cholecystokinin (CCK) is released by the mucosal cells in response to the presence of food in the upper intestine. As described in further detail below, human CCK is synthesized as a protoprotein of 115 amino acids. Active CCK forms are quickly taken into the blood through the digestive tract, and normally stimulate the secretion of enzymes by the acinar cells. However, stimulation of the CCK receptor by the CCK analogs cerulein and CCK-octapeptide (CCK-8) appears to lead to a worsening of morbidity and mortality in mammals in whom pancreatitis is induced. *See* Tani et al., *Pancreas* 5:284-290 (1990).

As indicated above, the digestive enzymes are synthesized as zymogens; proto-enzyme synthesis occurs in the rough endoplasmic reticulum of the acinar cells. The zymogens are then packaged within vesicles having a single lipid bilayer membrane. The zymogens are packed within the vesicles so densely that they appear as quasi-crystalline structures when observed under light microscopy and the zymogen granules are electron-dense when observed under the electron microscope. The vesicles are localized within the cytoplasm of the

acinar cells. Secretion of zymogens by the acinar cells occurs through vesicle docking and subsequent fusion with the plasma membrane, resulting in the liberation of the contents into the extracellular milieu.

Nerve cells appear to secrete neurotransmitters and other intercellular signaling factors through a mechanism of membrane fusion that is shared with other cell types, *see e.g.*, Rizo & Sudhof, *Nature Struct. Biol.* 5:839-842 (October 1998), hereby incorporated by reference herein, including the pancreatic acinar cells.

Although the Applicants do not wish to be bound by theory, it is believed that a vesicle first contacts the intracellular surface of the cellular membrane in a reaction called docking. Following the docking step the membrane fuses with and becomes part of the plasma membrane through a series of steps that currently remain relatively uncharacterized, but which clearly involve certain vesicle and membrane-associated proteins, as has been illustrated using neural models.

In neurons, neurotransmitters are packaged within synaptic vesicles, formed within the cytoplasm, then transported to the inner plasma membrane where the vesicles dock and fuse with the plasma membrane. Recent studies of nerve cells employing clostridial neurotoxins as probes of membrane fusion have revealed that fusion of synaptic vesicles with the cell membrane in nerve cells depends upon the presence of specific proteins that are associated with either the vesicle or the target membrane. *See id.* These proteins have been termed SNAREs. As discussed in further detail below, a protein alternatively termed synaptobrevin or VAMP

(vesicle-associated membrane protein) is a vesicle-associated SNARE (v-SNARE). There are at least two isoforms of synaptobrevin; these two isoforms are differentially expressed in the mammalian central nervous system, and are selectively associated with synaptic vesicles in neurons and secretory organelles in neuroendocrine cells. The target membrane-associated SNAREs (t-SNARES) include syntaxin and SNAP-25. Following docking, the VAMP protein forms a core complex with syntaxin and SNAP-25; the formation of the core complex appears to be an essential step to membrane fusion. See Rizo & Sudhof, *id.* and Neimmann et al., *Trends in Cell Biol.* 4:179-185 (May 1994), hereby incorporated by referenced herein.

Recently evidence has increasingly indicated that the SNARE system first identified in neural cells is a general model for membrane fusion in eukaryotic cells. A yeast exocytotic core complex similar to that of the synaptic vesicles of mammalian neural cells has been characterized, and found to contain three proteins: Sso 1 (syntaxin 1 homolog), SncI (synaptobrevin homolog), and sec9 (SNAP-25 homolog). Rizo & Sudhof, *id.* These proteins share a high degree of amino acid sequence homology with their mammalian synaptosomal counterparts.

All mammalian non-neuronal cells appear to contain cellubrevin, a synaptobrevin analog - this protein is involved in the intracellular transport of vesicles, and is cleaved by TeTx, BoNT/E, BoNT/F, and BoNT/G. Homologs of syntaxin have been identified in yeast (e.g., sso1p and sso2p) and mammalian non-neuronal cells (syn2p, syn3p, syn4p and syn5p). Finally, as

indicated above, a yeast SNAP-25 homolog, sec9 has been identified; this protein appears to essential for vesicle fusion with the plasma membrane.

Intoxication of neural cells by clostridial neurotoxins exploits specific characteristics of the SNARE proteins. These neurotoxins, most commonly found expressed in *Clostridium botulinum* and *Clostridium tetanus*, are highly potent and specific poisons of neural cells. These Gram positive bacteria secrete two related but distinct toxins, each comprising two disulfide-linked amino acid chains: a light chain (L) of about 50 KDa and a heavy chain (H) of about 100 KDa, which are wholly responsible for the symptoms of botulism and tetanus, respectively.

The tetanus and botulinum toxins are among the most lethal substances known to man; both toxins function by inhibiting neurotransmitter release in affected neurons. The tetanus neurotoxin (TeNT) acts mainly in the central nervous system, while botulinum neurotoxin (BoNT) acts at the neuromuscular junction; both toxins inhibit acetylcholine release from the nerve terminal of the affected neuron into the synapse, resulting in paralysis or reduced target organ function.

The tetanus neurotoxin (TeNT) is known to exist in one immunologically distinct type; the botulinum neurotoxins (BoNT) are known to occur in seven different immunologically distinct serotypes, termed BoNT/A through BoNT/G. While all of these latter types are produced by isolates of *C. botulinum*, two other species, *C. baratii* and *C. butyricum* also produce toxins similar to /F and /E, respectively. *See* e.g., Coffield et al., *The Site and Mechanism of Action of Botulinum*

Neurotoxin in *Therapy with Botulinum Toxin* 3-13 (Jankovic J. & Hallett M. eds. 1994), the disclosure of which is incorporated herein by reference.

Regardless of type, the molecular mechanism of intoxication appears to be similar. In the first step of the process, the toxin binds to the presynaptic membrane of the target neuron through a specific interaction between the heavy chain and a neuronal cell surface receptor; the receptor is thought to be different for each type of botulinum toxin and for TeNT. The carboxy terminal (C-terminal) half of the heavy chain is required for targeting of the toxin to the cell surface. The cell surface receptors, while not yet conclusively identified, appear to be distinct for each neurotoxin serotype.

In the second step, the toxin crosses the plasma membrane of the poisoned cell. The toxin is first engulfed by the cell through receptor-mediated endocytosis, and an endosome containing the toxin is formed. The toxin (or light chain thereof) then escapes the endosome into the cytoplasm of the cell. This last step is thought to be mediated by the amino terminal (N-terminal) half of the heavy chain, which triggers a conformational change of the toxin in response to a pH of about 5.5 or lower. Endosomes are known to possess a proton pump that decreases intra-endosomal pH. The conformational shift exposes hydrophobic residues in the toxin, which permits the toxin to embed itself in the endosomal membrane. The toxin then translocates through the endosomal membrane into the cytosol.

Either during or after translocation the disulfide bond joining the heavy and light chain is reduced, and

the light chain is released into the cytoplasm. The entire toxic activity of botulinum and tetanus toxins is contained in the light chain of the holotoxin; the light chain is a zinc (Zn++) endopeptidase which selectively cleaves the SNARE proteins essential for recognition and docking of neurotransmitter-containing vesicles with the cytoplasmic surface of the plasma membrane, and fusion of the vesicles with the plasma membrane. The light chain of TxNT, BoNT/B, BoNT/D, BoNT/F, and BoNT/G cause specific proteolysis of VAMP, an integral protein. During proteolysis, most of the VAMP present at the cytosolic surface of the synaptic vesicle is inactivated as a result of any one of these cleavage events. Each toxin cleaves a different specific peptide bond.

BoNT/A and /E selectively cleave the plasma membrane-associated SNARE protein SNAP-25; this protein is bound to and present on the cytoplasmic surface of the plasma membrane. BoNT/C1 cleaves syntaxin, which exists as an integral protein having most of its mass exposed to the cytosol. Syntaxin interacts with the calcium channels at presynaptic terminal active zones. *See* Tonello et al., *Tetanus and Botulism Neurotoxins in Intracellular Protein Catabolism* 251-260 (Suzuki K & Bond J. eds. 1996), the disclosure of which is incorporated by reference as part of this specification. Bo/NTC1 also appears to cleave SNAP-25.

Both TeNT and BoNT are specifically taken up by cells present at the neuromuscular junction. BoNT remains within peripheral neurons and, as indicated above, blocks release of the neurotransmitter acetylcholine from these cells.

By contrast TeNT, through its receptor, enters

vesicles that move in a retrograde manner along the axon to the soma, and is discharged into the intersynaptic space between motor neurons and the inhibitory neurons of the spinal cord. At this point, TeNT binds receptors of the inhibitory neurons, is again internalized, and the light chain enters the cytosol to block the release of the inhibitory neurotransmitters 4-aminobutyric acid (GABA) and glycine from these cells. Id.

International Patent Publication No. WO 96/33273 relates to derivatives of botulinum toxin designed to prevent neurotransmitter release from sensory afferent neurons to treat chronic pain. Such derivatives are targeted to nociceptive neurons using a targeting moiety that binds to a binding site of the surface of the neuron.

International Patent Publication No. 98/07864 discusses the production of recombinant toxin fragments that have domains that enable the polypeptide to translocate into a target cell or which increase the solubility of the polypeptide, or both.

Summary of the Invention

The present invention concerns methods and compositions useful for the treatment of acute pancreatitis. This condition is largely due to the defective secretion of zymogen granules by acinar cells, and by the premature co-mingling of the secreted zymogens with lysosomal hydrolysates capable of activating trypsin, thereby triggering the protease activation cascade and resulting in the destruction of

pancreatic tissue.

In one embodiment of this aspect, the invention is a therapeutic agent comprising a chimeric protein containing an amino acid sequence-specific endopeptidase activity which will specifically cleave at least one synaptic vesicle-associated protein selected from the group consisting of SNAP-25, syntaxin or VAMP, in combination with the translocation activity of the N-terminus of a clostridial neurotoxin heavy chain, wherein the chimeric protein further comprises a recognition domain which will bind a human cholecystokinin (CCK) receptor. Upon binding of the recognition domain of the protein to the CCK receptor, the protein is specifically transported into cells containing CCK receptors (pancreatic acinar cells) through receptor-mediated endocytosis. In a preferred embodiment, the CCK receptor is the CCK A receptor.

Once inside the acinar cell, the chimeric protein functions in a manner similar to that of a clostridial neurotoxin within its target neuron. The toxin moiety is translocated from the endosome into the cytoplasm, where it acts to cleave a SNARE protein identical or homologous to SNAP-25, syntaxin or VAMP. The cleavage of this protein prevents formation of a core complex between the SNARE proteins and thus prevents or reduces the extent of fusion of the vesicle with the target membrane. This, in turn, results in inhibition of zymogen release from the acinar cells and of zymogen activation by lysosomal hydrolases. The autodigestion of pancreatic tissue in acute pancreatitis is therefore reduced or eliminated.

Another embodiment of the present invention

concerns a method of treating a patient suffering from acute pancreatitis by administering an effective amount of such a chimeric protein.

Another embodiment of the invention concerns a therapeutic composition that contains the translocation activity of a clostridial neurotoxin heavy chain in combination with a recognition domain able to bind a specific cell type and a therapeutic element having an activity other than the endopeptidase activity of a clostridial neurotoxin light chain. A non-exclusive list of certain such therapeutic elements includes: hormones and hormone-agonists and antagonists, nucleic acids capable being of being used as replication, transcription, or translational templates (e.g., for expression of a protein drug having the desired biological activity or for synthesis of a nucleic acid drug as an antisense agent), enzymes, toxins, and the like.

In a preferred embodiment, the specific cell type is a pancreatic cell, most preferably a pancreatic acinar cell.

Another embodiment is drawn to methods for the treatment of acute pancreatitis comprising contacting an acinar cell with an effective amount of a composition comprising a chimeric protein containing an amino acid sequence-specific endopeptidase activity which will specifically cleave at least one synaptic vesicle-associated protein selected from the group consisting of SNAP-25, syntaxin or VAMP, in combination with the translocation activity of the N-terminus of a clostridial neurotoxin heavy chain, wherein the chimeric protein further comprises a recognition domain able to

bind to a cell surface protein characteristic of an human pancreatic acinar cell. Preferably the cell surface protein is a CCK receptor protein; most preferably the protein is the human CCK A protein. CCK receptors (CCK-A receptor and CCK-B receptor) are found mainly in on the surface of pancreatic acinar cells, although they are also found in some brain cells and, to a lesser extent on the surface of gastrointestinal cells.

Any suitable route of administration may be used in this aspect of the invention. Applicants currently prefer to administer the therapeutic agent in an intravenous infusion solution; however methods such as ingestion (particularly when associated with neurotoxin-associated proteins (NAPs); *see* Sharma et al., *J. Nat. Toxins* 7:239-253(1998), incorporated by reference herein), direct delivery to the pancreas, injection and the like may also be used. The agent is substantially specifically targeted to pancreatic cells; when the agent contains a CCK receptor-binding domain, the blood-brain barrier prevents the agent from interacting with brain cells.

In yet another embodiment the invention provides a composition comprising a drug or other therapeutic agent having an activity other than that of a clostridial neurotoxin light chain for intracellular delivery, said agent joined to the translocation domain of a clostridial neurotoxin heavy chain and a binding element able to recognize a cell surface receptor of a target cell. In a preferred embodiment, the target cell is not a neuron. Also, in this embodiment it is preferred that the drug or other therapeutic agent has an enzymatic,

catalytic, or other self-perpetuating mode of activity, so that the effective dose of drug is greater than the number of drug molecules delivered within the target cell. A non-exclusive list of certain such drugs would include: hormones and hormone-agonists and antagonists, nucleic acids capable being of being used as replication, transcription, or translational templates (e.g., for expression of a protein drug having the desired biological activity or for synthesis of a nucleic acid drug as an antisense agent), enzymes, toxins (such as diphtheria toxin or ricin), and the like.

In this embodiment the drug may be cleavably linked to the remainder of the composition in such a way as to allow for the release of the drug from the composition within the target cell.

The presently claimed compositions may be provided to the patient by intravenous administration, may be administered during surgery, or may be provided parenterally.

WO 95/32738, which shares ownership with the present application, describes transport proteins for the therapeutic treatment of neural cells. This application is incorporated by reference herein as part of this specification.

Detailed Description of the Preferred Embodiments

In a basic and presently preferred form, the invention comprises a therapeutic polypeptide comprising three features: a binding element, a translocation element, and a therapeutic element.

The binding element is able to bind to a specific target cell provided that the target cell is not a motor neuron or a sensory afferent neuron. Preferably, the binding element comprises an amino acid chain; also an independently, it is preferably located at or near the C-terminus of a polypeptide chain. By "binding element" is meant a chemical moiety able to preferentially bind to a cell surface marker characteristic of the target cell under physiological conditions. The cell surface marker may comprise a polypeptide, a polysaccharide, a lipid, a glycoprotein, a lipoprotein, or may have structural characteristics of more than one of these. By "preferentially interact" is meant that the disassociation constant (K_d) of the binding element for the cell surface marker is at least one order of magnitude less than that of the binding element for any other cell surface marker. Preferably, the disassociation constant is at least 2 orders of magnitude less, even more preferably the disassociation constant is at least 3 orders of magnitude less than that of the binding element for any other cell surface marker to which the therapeutic polypeptide is exposed. Preferably, the organism to be treated is a human.

In one embodiment the cell surface receptor comprises the histamine receptor, and the binding element comprises an variable region of an antibody which will specifically bind the histamine receptor.

In an especially preferred embodiment, the cell surface marker is a cholecystokinin (CCK) receptor. Cholecystokinin is a bioactive peptide that functions as both a hormone and a neurotransmitter in a wide variety of physiological settings. Thus, CCK is involved in the

regulation of gall bladder contraction, satiety, gastric emptying, and gut motility; additionally it is involved in the regulation of pancreatic exocrine secretion.

There are two types of CCK receptors, CCK A and CCK B; the amino acid sequences of these receptors have been determined from cloned cDNA. Despite the fact that both receptors are G protein-coupled receptors and share approximately 50% homology, there are distinct differences between their physiological activity. The CCK A receptor is expressed in smooth muscle cells of the gall bladder, smooth muscle and neurons within the gastrointestinal tract, and has a much greater affinity (>10^2 times higher) for CCK than the related peptide hormone gastrin. The CCK B receptor, found in the stomach and throughout the CNS, has roughly equal ability to bind CCK and gastrin.

The varied activities of CCK can be partly attributed to the fact that CCK is synthesized as procholecystokinin, a protoprotein of 115 amino acids, and is then post-translationally cleaved into a number of active fragments all sharing the same C-terminus. The amino acid sequence of human procholecystokinin is shown below; amino acid residues not present in the biologically active cleavage products are in lower case. All amino acid sequences herein are shown from N-terminus to C-terminus, unless expressly indicated otherwise:

Human procholecystokinin, having the amino acid sequence SEQ ID NO:1:

mnsgvclcvlmavlaagaltqpvppadpagsglqraeeaprrqlr VSQRT DGESRAHLGA LLARYIQQAR KAPSGRMSIV KNLQNLDPSH RISDRDYMGW MDF grrsaeeyeyps

Biologically active cleavage products of the full length CCK chain include:

CCK-58, having the amino acid sequence SEQ ID NO:2:

VSQRT DGESRAHLGA LLARYIQQAR KAPSGRMSIV KNLQNLDPSH RISDRDYMGW MDF;

CCK-39, having the amino acid sequence SEQ ID NO: 3:

YIQQAR KAPSGRMSIV KNLQNLDPSH RISDRDYMGW MDF;

CCK-33, having the amino acid sequence SEQ ID NO: 4:

KAPSGRMSIV KNLQNLDPSH RISDRDYMGW MDF;

CCK-12, having the amino acid sequence SEQ ID NO: 5:

ISDRDYMGW MDF;

and CCK-8, having the amino acid sequence SEQ ID NO: 6:

RDYMGW MDF.

In each case, the biologically active polypeptides contain post-translational modifications; in the case of

CCK species binding the CCK-A receptor, amidation of the C-terminal phenylalanine, and sulfatation of the tyrosine residue located seven residue from the C-terminus of the biologically active species are required for hoigh affinity binding ton the receptor. In the case of CCK-B, only the C-terminal amidation is necessary; sulfation of the tyrosine appears to make little diffrence in CCK-B binding. These modifications appear to be necessary for full biological activity, although both the unmodified C-terminal pentapeptide and tetrapeptide of CCK retains some biological activity. Kennedy et al., *J. Biol. Chem.* 272: 2920-2926 (1997), hereby incorporated by reference herein.

In a preferred embodiment, the biologically active therapeutic polypeptide of the present invention comprises a CCK binding element containing the post-translational modifications described above. This polypeptide can be produced by synthetic chemistry or, preferably, can be produced by a combination of recombinant and synthetic means using the "expressed protein ligation" (EPL) method. See Cotton & Muir, *Chemistry & Biology* 6:R247 (1999), hereby incorporated by reference herein. In this method the therapeutic polypeptide is expressed without the C-terminal binding element as a fusion protein with an "intein" polypeptide sequence positioned at the C-terminus thereof. The intein comprises a conserved cysteine, serine, or threonine residue at its amino terminus; the carboxyl terminus of the intein contains a functional binding sequence such as chitin binding domain (CBD), poly His (6 or more consecutive histidine residues), or another amino acid sequence capable of affinity binding. The

coding sequence of this recombinantly expressed polypeptide is constructed using standard recombinant DNA methods.

Additionally, standard solid phase peptide synthesis methods are employed to construct a synthetic peptide comprising a C-terminal amidated phenylalanine and the desired CCK amino acid sequence. Such methods are described in e.g., Bodansky, M. and Bodansky, A. *The Practice of Peptide Synthesis* (2d ed. Trost B.M., ed. Springer Laboratory 1994), hereby incorporated by reference herein. The synthetic peptide also contains an sulfated tyrosine at the position 7 residues from the carboxyl terminus. This can be done either by incorporation of commercially available Fmoc-Tyr(OSO_3^-)-OH into the peptide chain at the 7^{th} amino acid position prior to cleavage of the synthetic peptide from the solid support hereby incorporated by reference herein), or by standard peptide synthesis using tyrosine at position 7, followed by a sulfation reaction of the peptide resulting in tyrosine sulfate at the 7 position. See e.g., Koeller, K.M., *J. Am. Chem. Soc.* 122:742-743 (2000). The synthetic peptide is constructed with a cysteine (or serine or threonine) residue at the amino terminus.

It will be understood that one can use either hydroxyl-containing amino acids or cysteine as the amino terminal residue of the intein and the synthetic peptide, and either thiopheol, phenol or another nucleophile capable of creating a reactive ester or thioester linkage in accordance with the expressed protein ligation methods described herein. However,

thiol-containing amino acid residues and thipheonol or another sulfur-containing nucleophile are preferred.

Thus, according to one embodiment of the expressed protein ligation method, the fusion protein is immobilized following expression by incubation under selective binding conditions with a surface to which the binding partner of the carboxyl terminal has been joined (e.g., where the binding moiety is CBP, the surface may be a resin to which chitin is conjugated). The immobilized fusion protein is then permitted to react in a transthioesterification reaction with a S- or O-containing reagent (such as thiophenol or phenol) and the synthetic modified peptide described above. In this step, the intein which is joined to the carboxyl terminus of the therapeutic polypeptide is cleaved at the thioester (or ester) linkage, thus liberating the protein from the surface to which it was bound. The intein may be transiently replaced with the thiophenol group, and the resulting thioester is then itself attacked by the cysteine (or serine or threonine) residue of the synthetic peptide; this reaction is then spontaneously followed by a shift of the carbonyl bond from S (or O) to the N terminal nitrogen of the synthetic peptide, to form a peptide bond. The resultant therapeutic polypeptide thus comprises a threapeutic domain, a translocation domain, and a binding domain comprising a CCK sequence modified to contain the naturally occuring post-translational modifications.

As intended herein, the term "extein" refers to a portion of a chimeric polypeptide that borders one or more intein, and is subsequently ligated to either

another extein or a synthetic polypeptide in the EPL reaction referred to herein.

As intended herein, the term "intein" refers to a portion of a chimeric polypeptide containing an N-terminal cysteine, serine, or threonine which is excised from said polypeptide during the EPL reaction referred to herein.

Of course, the Applicants contemplate that this method of producing a CCK-containing therapeutic polypeptide is exemplary only, and that variations and modification of the above-described method will be well within the ability and knowledge of those of ordinary skill in the art in light of the present patent application.

While it will be understood that the applicants do not wish to be bound by theory, the following findings may assist an understanding the nature of the interaction between CCK and the CCK receptors, and thus between the CCK receptor binding element of an embodiment of the present invention and its CCK receptor target.

In pancreatic acinar cells the CCK A receptor undergoes internalization to intracellular sites within minutes after agonist exposure. Pohl et al., *J. Biol. Chem.* 272: 18179-18184 (1997), hereby incorporated by reference herein. The CCK B receptor has also shown the same ligand-dependant internalization response in transfected NIH 3T3 cells. In the CCK B receptor, but not the CCK A receptor, the endocytotic feature of the receptor been shown to be profoundly decreased by the deletion of the C terminal 44 amino acids of the

receptor chain, corresponding in both receptors to an cytoplasmic portion of the receptor chain.

Recent studies of the interaction between the CCK A receptor and CCK have shown that the primary receptor sequence region containing amino acid residues 38 through 42 is involved in the binding of CCK. Residues Trp_{39} and Gln_{40} appear to be essential for the binding of a synthetic CCK C-terminal nonapeptide (in which the methionine residues located at residue 3 and 6 from the C-terminus are substituted by norleucine and threonine respectively) to the receptor. Kennedy et al., *supra*. These residues do not appear to be essential for the binding of CCK analogs JMV 180 (corresponding the synthetic C-terminal heptapeptide of CCK in which the phenylalanylamide residue is substituted by a phenylethyl ester and the threonine is substituted with norleucine), and JMV 179 (in which the phenylalanylamide residue and the L-tryptophan residues of the synthetic CCK nonapeptide are substituted by a phenylethyl ester and D-tryptophan, respectively and the threonine is substituted with norleucine). *Id.*

These and similar studies have shed light on the structure of the CCK A receptor active site. Based on receptor binding experiments, a current structural model indicates that CCK residues Trp_{30} and Met_{31} (located at positions 4 and 3, respectively, from the C terminus of mature CCK-8) reside in a hydrophobic pocket formed by receptor residues Leu_{348}, Pro_{352}, Ile_{353} and Ile_{356}. CCK residue Asp_{32} (located at amino acid position 2 measured from the C terminus of CCK-8) seems to be involved in an ionic interaction with receptor residue Lys_{115}. CCK Tyr-

sulfate$_{27}$ (the CCK-8 residue 7 amino acids from C terminus) appears involved in an ionic interaction with receptor residue Lys$_{105}$ and a stacking interaction with receptor residue Phe$_{198}$. Ji, et al., 272 *J. Biol. Chem.* 24393-24401 (1997).

Such structural models provide detailed guidance to the person of ordinary skill in the art as to the construction of a variety of binding elements able to retain the binding characteristics of biologically active CCK peptides for the CCK-A receptor, for example, as, for example, by site directed mutagenesis of a clostridial neurotoxin heavy chain. Similarly, models deduced using similar methodologies have been proposed for the CCK B receptor, see e.g., Jagerschmidt, A. et al., *Mol. Pharmacol.* 48:783-789 (1995), and can be used as a basis for the construction of binding elements that retain binding characteristics similar to the CCK B receptor.

It will be appreciated that the CCK-B receptor is known to exist on the surface of neurons associated with the certal nervious system. In one alternative embodiment of the present invention the therapeutic polypeptide may be directed (for example, by intrathecal application) to these neurons rather than to the pancreas); in such a case, the binding element may comprise a CCK containing the C terminal amidation only. Such a binding element may be constructed using the expressed protein ligation (EPL) methods described above. Indeed, EPL methods may be used to introduce and desired or required modifications to the therapeutic element, the translocation element, and/or the binding element of the claimed therapeutic polypeptide.

Additionally, the binding element may comprise a variable region of an antibody which will bind the CCK-A or CCK-B receptor.

Nucleic acids encoding polypeptides containing such a binding element may be constructed using molecular biology methods well known in the art; see e.g., Sambrook et al., *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Laboratory Press 2d ed. 1989), and expressed within a suitable host cell. The disclosure of this latter reference is incorporated by reference herein in its entirety.

The translocation element comprises a portion of a clostridial neurotoxin heavy chain having a translocation activity. By "translocation" is meant the ability to facilitate the transport of a polypeptide through a vesicular membrane, thereby exposing some or all of the polypeptide to the cytoplasm.

In the various botulinum neurotoxins translocation is thought to involve an allosteric conformational change of the heavy chain caused by a decrease in pH within the endosome.

This conformational change appears to involve and be mediated by the N terminal half of the heavy chain and to result in the formation of pores in the vesicular membrane; this change permits the movement of the proteolytic light chain from within the endosomal vesicle into the cytoplasm. See e.g., Lacy, et al., *Nature Struct. Biol.* 5:898-902 (October 1998).

The amino acid sequence of the translocation-mediating portion of the botulinum neurotoxin heavy chain is known to those of skill in the art; additionally, those amino acid residues within this

portion that are known to be essential for conferring the translocation activity are also known.

It would therefore be well within the ability of one of ordinary skill in the art, for example, to employ the naturally occurring N-terminal peptide half of the heavy chain of any of the various *Clostridium tetanus* or *Clostridium botulinum* neurotoxin subtypes as a translocation element, or to design an analogous translocation element by aligning the primary sequences of the N-terminal halves of the various heavy chains and selecting a consensus primary translocation sequence based on conserved amino acid, polarity, steric and hydrophobicity characteristics between the sequences. The therapeutic element of the present invention may comprise, without limitation: active or inactive (i.e., modified) hormone receptors (such as androgen, estrogen, retinoid, perioxysome proliferator and ecdysone receptors etc.), and hormone-agonists and antagonists, nucleic acids capable being of being used as replication, transcription, or translational templates (e.g., for expression of a protein drug having the desired biological activity or for synthesis of a nucleic acid drug as an antisense agent), enzymes, toxins (including apoptosis-inducing agents), and the like.

In a preferred embodiment, the therapeutic element is a polypeptide comprising a clostridial neurotoxin light chain or a portion thereof retaining the SNARE-protein sequence-specific endopeptidase activity of a clostridial neurotoxin light chain. The amino acid sequences of the light chain of botulinum neurotoxin (BoNT) subtypes A-G have been determined, as has the

amino acid sequence of the light chain of the tetanus neurotoxin (TeNT). Each chain contains the Zn^{++}-binding motif **His-Glu-x-x-His** (N terminal direction at the left) characteristic of Zn^{++}-dependent endopeptidases (HELIH in TeNT, BoNT/A /B and /E; HELNH in BoNT/C; and HELTH in BoNT/D).

Recent studies of the BoNT/A light chain have revealed certain features important for the activity and specificity of the toxin towards its target substrate, SNAP-25. Thus, studies by Zhou et al. *Biochemistry* 34:15175-15181 (1995) have indicated that when the light chain amino acid residue His_{227} is substituted with tyrosine, the resulting polypeptide is unable to cleave SNAP-25; Kurazono et al., *J. Biol. Chem.* 14721-14729 (1992) performed studies in the presynaptic cholinergic neurons of the buccal ganglia of *Aplysia californica* using recombinant BoNT/A light chain that indicated that the removal of 10 N-terminal or 32 C-terminal residues did not abolish toxicity, but that removal of 10 N-terminal or 57 C-terminal residues abolished toxicity in this system. Most recently, the crystal structure of the entire BoNT/A holotoxin has been solved; the active site is indicated as involving the participation of His_{222}, Glu_{223}, His_{226}, Glu_{261} and Tyr_{365}. Lacy et al., *supra*. (These residues correspond to His_{223}, Glu_{224}, His_{227}, Glu_{262} and Tyr_{366} of the BoNT/A L chain of Kurazono et al., *supra*.) Interestingly, an alignment of BoNT/A through E and TeNT light chains reveals that every such chain invariably has these residues in positions analogous to BoNT/A. Kurazono et al., *supra*.

The catalytic domain of BoNT/A is very specific for the C-terminus of SNAP-25 and appears to require a minimum of 16 SNAP-25 amino acids for cleavage to occur. The catalytic site resembles a pocket; when the light chained is linked to the heavy chain via the disulfide bond between Cys_{429} and Cys_{453}, the translocation domain of the heavy chain appears to block access to the catalytic pocket until the light chain gains entry to the cytosol. When the disulfide bond is reduced, the two polypeptide chains dissociate, and the catalytic pocket is then "opened" and the light chain is fully active.

As described above, VAMP and syntaxin are cleaved by BoNT/B, D, F, G and TeNT, and BoNT/C_1, respectively, while SNAP-25 is cleaved by BoNT/A and E.

The substrate specificities of the various clostridial neurotoxin light chains other than BoNT/A are known. Therefore, the person of ordinary skill in the art could easily determine the toxin residues essential in these subtypes for cleavage and substrate recognition (for example, by site-directed mutagenesis or deletion of various regions of the toxin molecule followed by testing of proteolytic activity and substrate specificity), and could therefore easily design variants of the native neurotoxin light chain that retain the same or similar activity.

Additionally, construction of the therapeutic agents set forth in this specification would be easily constructed by the person of skill in the art. It is well known that the clostridial neurotoxins have three functional domains analogous to the three elements of the present invention. For example, and without

limitation, the BoNT/A neurotoxin light chain is present in amino acid residues 1-448 of the BoNT/A prototoxin (i.e., before nicking of the prototoxin to form the disulfide-linked dichain holotoxin); this amino acid sequence is provided below as SEQ ID NO: 7. Active site residues are underlined:

BoNT/A light chain (SEQ ID NO:7)

MPFVNKQFNYKDPVNGVDIAYIKIPNAGQMQPVKAFKIHNKIWV
IPERDTFTNPEEGDLNPPPEAKQVPVSYYDSTYLSTDNEKDNYLKGVTKLFERIYSTD
LGRMLLTSIVRGIPFWGGSTIDTELKVIDTNCINVIQPDGSYRSEELNLVIIGPSADI
IQFECKSFGHEVLNLTRNGYGSTQYIRFSPDFTFGFEESLEVDTNPLLGAGKFATDPA
VTLAHELIHAGHRLYGIAINPNRVFKVNTNAYYEMSGLEVSFEELRTFGGHDAKFIDS
LQENEFRLYYYNKFKDIASTLNKAKSIVGTTASLQYMKNVFKEKYLLSEDTSGKFSVD
KLKFDKLYKMLTEIYTEDNFVKFFKVLNRKTYLNFDKAVFKINIVPKVNYTIYDGFNL
RNTNLAANFNGQNTEINNMNFTKLKNFTGLFEFYKLLCVRGIITSKTKSLDKGYNK;

The heavy chain N-terminal (H_N) translocation domain is contained in amino acid residues 449-871 of the BoNT/A amino acid sequence, shown below as SEQ ID NO: 8; a gated ion channel-forming domain probably essential for the translocation activity of this peptide is underlined (see Oblatt-Montal et al., *Protein Sci.* 4:1490-1497(1995), hereby incorporated by reference herein.

ALNDLCIKVNNWDLFFSPSEDNFTNDLNKGEEITSDTNIEAAEENISLDLIQQYYLTFNF
DNEPENISIENLSSDIIGQLELMPNIERFPNGKKYELDKYTMFHYLRAQEFEHGKSRI
ALTNSVNEALLNPSRVYTFFSSDYVKKVNKATEAAMFLGWVEQLVYDFTDETSEVSTT
DKIADITIIIPYIGPALNIGNMLYKDDFVGALIFSGAVILLEFIPEIAIPVLGTFALV
SYIANKVLTVQTIDNALSKRNEKWDEVYKYIVTNWLAKVNTQIDLIRKKMKEALENQA
EATKAIINYQYNQYTEEEKNNINFNIDDLSSKLNESINKAMININKFLNQCSVSYLMN
SMIPYGVKRLEDFDASLKDALLKYIYDNRGTLIGQVDRLKDKVNNTLSTDIPFQLSKY
VDNQRLLSTFTEYIK;

The heavy chain C-terminal neural cell binding domain is contained in amino acid residues 872-1296 (SEQ ID NO: 9) of the BoNT/A prototoxin.

NIINTSILNLRYESNHLIDLSRYASKINIGSKVNFDPIDKNQI
QLFNLESSKIEVILKNAIVYNSMYENFSTSFWIRIPKYFNSISLNNEYTIINCMENNS
GWKVSLNYGEIIWTLQDTQEIKQRVVFKYSQMINISDYINRWIFVTITNNRLNNSKIY
INGRLIDQKPISNLGNIHASNNIMFKLDGCRDTHRYIWIKYFNLFDKELNEKEIKDLY
DNQSNSGILKDFWGDYLQYDKPYYMLNLYDPNKYVDVNNVGIRGYMYLKGPRGSVMTT
NIYLNSSLYRGTKFIIKKYASGNKDNIVRNNDRVYINVVVKNKEYRLATNASQAGVEK
ILSALEIPDVGNLSQVVVMKSKNDQGITNKCKMNLQDNNGNDIGFIGFHQFNNIAKLV
ASNWYNRQIERSSRTLGCSWEFIPVDDGWGERPL

The amino acid sequence of the BoNT/A prototoxin is encoded by nucleotides 358 to 4245 of the neurotoxin cDNA sequence, set forth herein below as SEQ ID NO: 10.

aagcttctaa atttaaatta ttaagtataa atccaaataa acaatatgtt caaaaacttg
atgaggtaat aatttctgta ttagataata tggaaaaata tatagatata tctgaagata
atagattgca actaatagat aacaaaaata acgcaaagaa gatgataatt agtaatgata
tatttatttc caattgttta accctatctt ataacggtaa atatatatgt ttatctatga
aagatgaaaa ccataattgg atgatatgta ataatgatat gtcaaagtat ttgtatttat
ggtcatttaa ataattaata atttaattaa ttttaaatat tataagaggt gttaaatatg
ccatttgtta ataaacaatt taattataaa gatcctgtaa atggtgttga tattgcttat
ataaaaattc caaatgcagg acaaatgcaa ccagtaaaag cttttaaaat tcataataaa
atatgggtta ttccagaaag agatacattt acaaatcctg aagaaggaga tttaaatcca
ccaccagaag caaaacaagt tccagtttca tattatgatt caacatattt aagtacagat
aatgaaaaag ataattattt aaagggagtt acaaaattat ttgagagaat ttattcaact
gatcttggaa gaatgttgtt aacatcaata gtaaggggaa taccattttg gggtggaagt
acaatagata cagaattaaa agttattgat actaattgta ttaatgtgat acaaccagat
ggtagttata gatcagaaga acttaatcta gtaataatag gaccctcagc tgatattata

cagtttgaat gtaaaagctt tggacatgaa gttttgaatc ttacgcgaaa tggttatggc
tctactcaat acattagatt tagcccagat tttacatttg gttttgagga gtcacttgaa
gttgatacaa atcctctttt aggtgcaggc aaatttgcta cagatccagc agtaacatta
gcacatgaac ttatacatgc tggacataga ttatatggaa tagcaattaa tccaaatagg
gtttttaaag taaatactaa tgcctattat gaaatgagtg ggttagaagt aagctttgag
gaacttagaa catttggggg acatgatgca aagtttatag atagtttaca ggaaaacgaa
tttcgtctat attattataa taagtttaaa gatatagcaa gtacacttaa taaagctaaa
tcaatagtag gtactactgc ttcattacag tatatgaaaa atgtttttaa agagaaatat
ctcctatctg aagatacatc tggaaaattt tcggtagata aattaaaatt tgataagtta
tacaaaatgt taacagagat ttacacagag gataattttg ttaagttttt taaagtactt
aacagaaaaa catatttgaa ttttgataaa gccgtattta agataaatat agtacctaag
gtaaattaca caatatatga tggatttaat ttaagaaata caaatttagc agcaaacttt
aatggtcaaa atacagaaat taataatatg aattttacta aactaaaaaa ttttactgga
ttgtttgaat tttataagtt gctatgtgta agagggataa taacttctaa aactaaatca
ttagataaag gatacaataa ggcattaaat gatttatgta tcaaagttaa taattgggac
ttgtttttta gtccttcaga agataatttt actaatgatc taaataaagg agaagaaatt
acatctgata ctaatataga agcagcagaa gaaaatatta gtttagattt aatacaacaa
tattatttaa cctttaattt tgataatgaa cctgaaaata tttcaataga aaatctttca
agtgacatta taggccaatt agaacttatg cctaatatag aaagatttcc taatggaaaa
aagtatgagt tagataaata tactatgttc cattatcttc gtgctcaaga atttgaacat
ggtaaatcta ggattgcttt aacaaattct gttaacgaag cattattaaa tcctagtcgt
gtttatacat ttttttcttc agactatgta aagaaagtta ataaagctac ggaggcagct
atgtttttag gctgggtaga acaattagta tatgatttta ccgatgaaac tagcgaagta
agtactacgg ataaaattgc ggatataact ataattattc catatatagg acctgcttta
aatataggta atatgttata taaagatgat tttgtaggtg ctttaatatt ttcaggagct
gttattctgt tagaatttat accagagatt gcaatacctg tattaggtac ttttgcactt

gtatcatata ttgcgaataa ggttctaacc gttcaaacaa tagataatgc tttaagtaaa
agaaatgaaa aatgggatga ggtctataaa tatatagtaa caaattggtt agcaaaggtt
aatacacaga ttgatctaat aagaaaaaaa atgaaagaag ctttagaaaa tcaagcagaa
gcaacaaagg ctataataaa ctatcagtat aatcaatata ctgaggaaga gaaaaataat attaatttta atattgatga tttaagttcg aaacttaatg agtctataaa taaagctatg attaatataa ataaattttt gaatcaatgc tctgtttcat atttaatgaa ttctatgatc
ccttatggtg ttaaacggtt agaagatttt gatgctagtc ttaaagatgc attattaaag
tatatatatg ataatagagg aactttaatt ggtcaagtag atagattaaa agataaagtt
aataatacac ttagtacaga tatacctttt cagctttcca aatacgtaga taatcaaaga
ttattatcta catttactga atatattaag aatattatta atacttctat attgaattta
agatatgaaa gtaatcattt aatagactta tctaggtatg catcaaaaat aaatattggt
agtaaagtaa attttgatcc aatagataaa aatcaaattc aattatttaa tttagaaagt
agtaaaattg aggtaatttt aaaaaatgct attgtatata atagtatgta tgaaaatttt
agtactagct tttggataag aattcctaag tattttaaca gtataagtct aaataatgaa
tatacaataa taaattgtat ggaaaataat tcaggatgga aagtatcact taattatggt
gaaataatct ggactttaca ggatactcag gaaataaaac aaagagtagt ttttaaatac
agtcaaatga ttaatatatc agattatata aacagatgga tttttgtaac tatcactaat
aatagattaa ataactctaa aatttatata aatggaagat taatagatca aaaaccaatt
tcaaatttag gtaatattca tgctagtaat aatataatgt ttaaattaga tggttgtaga
gatacacata gatatatttg gataaaatat tttaatcttt ttgataagga attaaatgaa
aaagaaatca aagatttata tgataatcaa tcaaattcag gtattttaaa agacttttgg
ggtgattatt tacaatatga taaaccatac tatatgttaa atttatatga tccaaataaa
tatgtcgatg taaataatgt aggtattaga ggttatatgt atcttaaagg gcctagaggt
agcgtaatga ctacaaacat ttatttaaat tcaagtttgt atagggggac aaaatttatt
ataaaaaaat atgcttctgg aaataaagat aatattgtta gaaataatga tcgtgtatat
attaatgtag tagttaaaaa taaagaatat aggttagcta ctaatgcatc acaggcaggc
gtagaaaaaa tactaagtgc attagaaata cctgatgtag gaaatctaag tcaagtagta

gtaatgaagt caaaaaatga tcaaggaata acaaataaat gcaaaatgaa tttacaagat
aataatggga atgatatagg ctttatagga tttcatcagt ttaataatat agctaaacta
gtagcaagta attggtataa tagacaaata gaaagatcta gtaggacttt gggttgctca
tgggaattta ttcctgtaga tgatggatgg ggagaaaggc cactgtaatt aatctcaaac
tacatgagtc tgtcaagaat tttctgtaaa catccataaa aattttaaaa ttaatatgtt
taagaataac tagatatgag tattgtttga actgcccctg tcaagtagac aggtaaaaaa
ataaaaatta agatactatg gtctgatttc gatattctat cggagtcaga ccttttaact
tttcttgtat ccttttttgta ttgtaaaact ctatgtattc atcaattgca agttccaatt
agtcaaaatt atgaaacttt ctaagataat acatttctga ttttataatt tcccaaaatc
cttccatagg accattatca atacatctac caactcgaga catactttga gttgcgccta
tctcattaag tttattcttg aaagatttac ttgtatattg aaaaccgcta tcactgtgaa
aaagtggact agcatcagga ttggaggtaa ctgctttatc aaaggtttca aagacaagga
cgttgttatt tgattttcca agtacatagg aaataatgct attatcatgc aaatcaagta
tttcactcaa gtacgccttt gtttcgtctg ttaac

Of course, three distinct domains analogous to those described above for BoNT/A exist for all the BoNT subtypes as well as for TeNT neurotoxin; an alignment of the amino acid sequences of these holotoxins will reveal the sequence coordinates for these other neurotoxin species. Additionally, while sequence information is given above for BoNT/A, the amino acid sequences of all BoNT species and tetanus toxin TeNT are known and can easily be obtained from, for example, the NCBI Gen-Bank Web site: www.ncbi.nlm.nih.gov. The Clostrdial neurotoxin nucleotide and amino acid sequences disclosed at this site are expressly incorporated by reference herein.

Preferably, the translocation element and the binding element of the compositions of the present invention are separated by a spacer moiety that facilitates the binding element's binding to the desired cell surface receptor. Such a spacer may comprise, for example, a portion of the BoNT Hc sequence (so long as the portion does not retain the ability to bind to the BoNT or TeNT binding site of motor neurons or sensory afferent neurons), another sequence of amino acids, or a hydrocarbon moiety. The spacer moiety may also comprise a proline, serine, threonine and/or cysteine-rich amino acid sequence similar or identical to a human immunoglobulin hinge region. In a preferred embodiment, the spacer region comprises the amino acid sequence of an immunoglobulin γ1 hinge region; such a sequence has the sequence (from N terminus to C terminus):

EPKSCDKTHTCPPCP (SEQ ID NO:11)

It will be understood that none of the examples or embodiments described herein are to be construed as limiting the scope of the invention, which is defined solely by the claims that conclude this specification.

Example 1:

An agent for the treatment of acute pancreatitis is constructed as follows.

A culture of *Clostridium botulinum* is permitted to grown to confluence. The cells are then lysed and total RNA is extracted according to conventional methods and in the presence of an RNAse inhibitor. The RNA preparation is then passed over a oligo(dT) cellulose column, the polyadenylated messenger RNA is permitted to

bind, and the column is washed with 5-10 column volumes of 20 mM Tris pH 7.6, 0.5 M NaCl, 1 mM EDTA (ethylenediamine tetraacetic acid), 0.1% (w/v) SDS (sodium dodecyl sulfate). Polyadenylated RNA is then eluted with 2-3 column volumes of STE (10 mM Tris (pH 7.6), 1 mM EDTA, 0.05% (w/v) SDS). The pooled mRNA is then precipitated in 2 volumes of ice cold ethanol, pelleted in a centrifuge at 10,000 x g for 15 minutes, then redissolved in a small volume of STE.

The BoNT/A mRNA is used as a template for DNA synthesis using Moloney murine leukemia virus reverse transcriptase (MMLV-RT), then the L chain and then H_N chain of the neurotoxin is amplified from the cDNA by the polymerase chain reaction (PCR) using appropriate oligonucleotide primers whose sequences are designed based on the BoNT/A neurotoxin cDNA sequence of SEQ ID NO: 9. These procedures are performed using the standard techniques of molecular biology as detailed in, for example, Sambrook et al., already incorporated by reference herein. The primer defining the beginning of the coding region (5'side of the L chain fragment) is given a StuI site. The PCR primer defining the 3' end of the H_N-encoding domain has the following features (from 3' to 5'): a 5' region sufficiently complementary to the 3' end of the H_N-encoding domain to anneal thereto under amplification conditions, a nucleotide sequence encoding the human immunoglobulin hinge region γ_1 (SEQ ID NO:11), a nucleotide sequence encoding the human CCK-8 octapeptide (SEQ ID NO:6), and a unique restriction endonuclease cleavage site.

The PCR product (termed BoNT/A$^{L\text{-}HN\text{-}\gamma\text{-}CCK}$) is purified by agarose gel electrophoresis, and cloned into a pBluescript II SK vector. The resulting plasmid is used to transform competent *E. coli* cells, and a preparation of the resulting plasmid is made. The BoNT/A$^{L\text{-}HN\text{-}\gamma\text{-}CCK}$ fragment is excised from the pBluescript vector and cloned into a mammalian expression vector immediately downstream of a strong promoter. The resulting vector is used to transfect a culture of the appropriate host cell, which is then grown to confluence. Expression of the BoNT/A$^{L\text{-}HN\text{-}\gamma\text{-}CCK}$ polypeptide is induced, and the cells are lysed. The polypeptide is first purified by gel exclusion chromatography, the fractions containing the recombinant therapeutic agent are pooled, then the BoNT/A$^{L\text{-}HN\text{-}\gamma\text{-}CCK}$ polypeptide is further purified using an anti-Ig affinity column wherein the antibody is directed to the γ_1 hinge region of a human immunoglobulin.

Example 2: Method of Treating a Patient Suffering from Acute Pancreatitis

A therapeutically effective amount of the BoNT/A$^{L\text{-}HN\text{-}\gamma CCK}$ agent constructed and purified as set forth in Example 1 is formulated in an acceptable infusion solution. Properties of pharmacologically acceptable infusion solutions, including proper electrolyte balance, are well known in the art. This solution is provided intravenously to a patient suffering from acute pancreatitis on a single day over a period of one to two hours. Additionally, the patient is fed intravenously on a diet low in complex carbohydrates, complex fats and proteins.

At the beginning of treatment, the patient's pancreas shows signs of autodigestion, as measured by blood amylase levels. After the treatment regimen, autodigestion has ceased, and the patient's pancreas has stabilized.

Example 3: Alternative Treatment Method

In this example, a patient suffering from acute pancreatitis is treated as in Example 2, with, the therapeutic agent given continuously over a period of two weeks. After the treatment regimen, autodigestion has ceased, and the patient's pancreas has stabilized.

Example 4: Alternative Treatment Method

In this example, a patient suffering from acute pancreatitis is given a single pharmacologically effective amount of the therapeutic agent of Example 1 by parenteral administration. Two days after the treatment regimen, autodigestion has ceased and the patient's pancreas has stabilized.

It will be understood that the present invention is not to be limited by the embodiments and examples described herein, and that the invention is defined solely by the claims that conclude this specification.